

# Failure to Precondition Pathological Human Myocardium

Sudip Ghosh, FRCS,\* Nicholas B. Standen, PhD,† Manuel Galiñanes, MD, PhD\*

Leicester, United Kingdom

<b>OBJECTIVES</b>	We investigated the effects of ischemic preconditioning (PC) on diabetic and failing human myocardium and the role of mitochondrial $K_{ATP}$ channels on the response in these diseased tissues.
<b>BACKGROUND</b>	There is conflicting evidence to suggest that PC is a healthy heart phenomenon.
<b>METHODS</b>	Right atrial appendages were obtained from seven different groups of patients: nondiabetics, diet-controlled diabetics, noninsulin-dependent diabetics (NIDD) receiving $K_{ATP}$ channel blockers, insulin-dependent diabetics (IDD), and patients with left ventricular ejection fraction (LVEF) >50%, LVEF between 30% and 50% and LVEF <30%. After stabilization, the muscle slices were randomized into five experimental groups (n = 6/group): 1) aerobic control—incubated in oxygenated buffer for 210 min, 2) ischemia alone—90 min ischemia followed by 120 min reoxygenation, 3) preconditioning by 5 min ischemia/5 min reoxygenation before 90 min ischemia/120 min reoxygenation, 4) diazoxide (Mito $K_{ATP}$ opener, 0.1 mm)—for 10 min before the 90 min ischemia/120 min reoxygenation and 5) glibenclamide (10 $\mu$ m)—10 min exposure prior to PC (only in the diabetic patient groups). Creatine kinase leakage into the medium (CK, U/g wet wt) and MTT reduction (OD/mg wet wt), an index of cell viability, were assessed at the end of the experiment.
<b>RESULTS</b>	Ischemia caused similar injury in both normal and diseased tissue. Preconditioning prevented the effects of ischemia in all groups except NIDD, IDD and poor cardiac function (<30%). In the diazoxide-treated groups, protection was mimicked in all groups except the NIDD and IDD groups. Interestingly, glibenclamide abolished protection in nondiabetic and diet-controlled NIDD groups and did not affect NIDD groups receiving $K_{ATP}$ channel blockers or IDD groups.
<b>CONCLUSIONS</b>	These results show that failure to precondition the diabetic heart is due to dysfunction of the mitochondrial $K_{ATP}$ channels and that the mechanism of failure in the diabetic heart lies in elements of the signal transduction pathway different from the mitochondrial $K_{ATP}$ channels. (J Am Coll Cardiol 2001;37:711–8) © 2001 by the American College of Cardiology

Ischemic preconditioning (PC) falls within a spectrum of adaptive responses to ischemia and represents the ability of the myocardium to adapt to sublethal ischemic stress in the short term so that it is more resistant to a subsequent, potentially injurious period of ischemia. Preconditioning consists of two phases of protection: an early or first window of protection ( $\leq 2$  h) and a delayed or second window of protection ( $\geq 24$  h). The underlying mechanism of PC has been extensively investigated; however, the basis of such cardioprotection is not fully elucidated. The most favored hypothesis for the first window of PC suggests that a variety of endogenous ligands such as adenosine, bradykinin, catecholamines and opioids activate receptors linked to protein kinase C (PKC) to initiate an intracellular signal transduction pathway. Protein kinase C may activate a tyrosine kinase, which in turn activates mitogen-activated protein or c-Jun-N-terminal kinases (JNK) kinases leading to phosphorylation of  $K_{ATP}$  channels (1). There is convincing evidence in the literature that  $K_{ATP}$  channels are involved in

the protection of ischemic PC in the human myocardium (2,3), although the exact place of these channels in the signal transduction pathway is still unclear (4–6). Recently we have shown that the mitochondrial, not the sarcolemmal,  $K_{ATP}$  channel is responsible for this powerful protective mechanism in the human myocardium (7).

Within the enormous amount of research describing the cellular basis of the PC response, relatively few studies relevant to coronary artery disease in humans have focused on the effect of PC in hearts with concurrent abnormalities. More importantly, even amongst those studies, the conclusions have been conflicting. Clinical studies identify a number of conditions that increase mortality from myocardial infarction; these include heart failure, diabetes, hypertension, aging and hypercholesterolemia (8,9). It is plausible that these conditions interfere with the biochemical pathways underlying the PC response.

Cardiovascular disease associated with diabetes mellitus is a major cause of death in patients with diabetes (10). In the vast majority of animal studies, diabetic hearts demonstrate a reduced tolerance to anoxia, hypoxia or ischemia (11–13), but studies that have investigated the effect of preconditioning on diabetic hearts have yielded confusing data. Tosaki et al. (14) have shown in the streptozotocin-induced diabetic rat heart that PC does not confer cardiac protection. Their results were opposed to those of Liu et al. (15), who had

From the \*Division of Cardiac Surgery, Department of Surgery and the †Department of Cell Physiology and Pharmacology, University Hospitals Leicester, Glenfield Campus, Leicester, United Kingdom. Presented at the 72nd Annual AHA Scientific Congress, November 7–10, 1999, Atlanta, Georgia. This study was supported in part by a grant from the University of Leicester.

Manuscript received June 6, 2000; revised manuscript received September 28, 2000, accepted November 3, 2000.

#### Abbreviations and Acronyms

CK	= creatine kinase
DCD	= diet-controlled diabetes
IDD	= insulin-dependent diabetes
K <sub>ATP</sub>	= ATP-dependent potassium channels
LVEF	= left ventricular ejection fraction
MTT	= 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
NIDD	= noninsulin-dependent diabetes
PC	= preconditioning
PKC	= protein kinase C

earlier shown, also in the rat heart, that myocardial infarction is reduced in diabetes and that PC further increases the protection of these hearts. There are very few studies in human diabetic tissue. Cleveland et al. (16) used a functional isolated atrial trabeculae model and showed that PC did not confer any protection of the myocardium on patients taking long-term oral hypoglycemic agents. They hypothesized that long-term inhibition of K<sub>ATP</sub> channels with these agents may be responsible for the excess cardiovascular mortality associated with diabetes.

Heart failure is common in all forms of heart disease. Mechanical dysfunction of the failing heart is due to many factors, including neurohormonal disturbance, accumulation of extracellular matrix, alteration of excitation-contraction coupling and maladaptation of myocardial energetics (17). Very few studies have investigated the effects of the PC response in the failing myocardium in light of alterations in the cellular metabolic and biochemical pathways associated with heart failure. Cleveland et al. (3) showed in isolated ventricular trabeculae from patients requiring heart transplantation that PC conferred protection. However, more recently Dekker et al. (18) have studied perfused papillary muscles from rabbits in which cardiac failure has been induced by a combination of pressure and volume overload-

ing. The end points used to assess responses to ischemia, namely the time to onset of a rise in the intracellular concentration of Ca<sup>2+</sup> (cellular uncoupling) and of ischemic contracture, were delayed by PC in normal myocardium but were exaggerated by PC in the failing myocardium.

The aims of the present study were 1) to investigate the effects of PC on the diabetic and failing human myocardium, and 2) to investigate the role of mitochondrial K<sub>ATP</sub> channels in the responses of these pathological conditions. These studies were carried out in an in vitro model of human right atrial myocardium of simulated ischemia and reoxygenation.

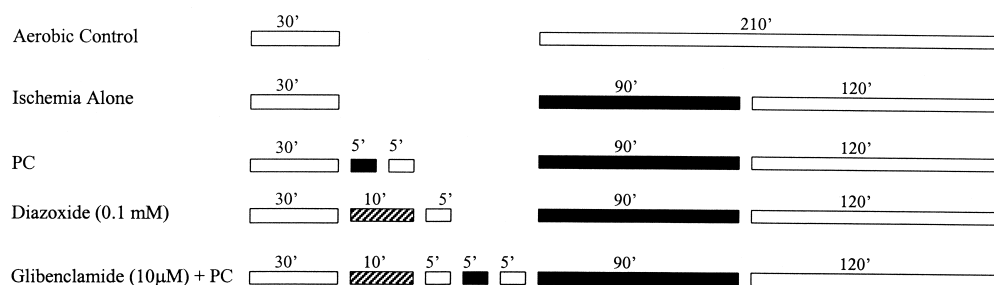
## MATERIALS AND METHODS

**Experimental preparation.** The right atrial appendage of patients undergoing elective coronary artery surgery or aortic valve replacement was obtained. Patients were excluded if they had enlarged right atriums, atrial arrhythmias or right ventricular failure, or were taking opioid analgesia. Patient characteristics are detailed in Table 1. Local ethical committee approval was obtained for the harvesting technique, and the investigation conforms to the principles outlined in the Declaration of Helsinki. The specimens were collected in oxygenated HEPES buffered solution at 4° to 5°C and immediately sectioned and prepared for study. Briefly, the appendage was mounted onto a ground glass plate with the epicardial surface face down and was then sliced with surgical skin graft blades (Shwann-Morton, Sheffield, United Kingdom) to a thickness of between 300 and 500 μm. The specimen and the slide were kept moist throughout the procedure. Then 30 to 50 mg of muscle were transferred to conical flasks (25 ml Erlenmeyer flasks, Duran, Astell Scientific, Kent, United Kingdom) containing 10 ml of oxygenated buffered solution. Following this, the flasks were placed in a shaking water bath maintained at

**Table 1.** Patient Characteristics

	Nondiabetics	DCD	NIDD	IDD	LVEF >50%	LVEF = 30%-50%	LVEF <30%
Number	6	6	6	6	6	6	6
Age (yrs ± SEM)	68.1 ± 3.2	65.1 ± 1.9	59.4 ± 5.4	70.2 ± 2.8	58.7 ± 9.8	66.1 ± 2.8	69.4 ± 5.9
Gender (male/female)	4/2	3/3	5/1	4/2	3/3	2/4	1/5
Hypertension (%)	50	33	50	67	33	33	50
Previous MI (%)	0	33	33	33	0	0	83
Medication							
Glibenclamide			3				
Gliclazide			2				
Tolbutamide			1				
Nitrates	6	6	5	5	6	6	5
Ca <sup>2+</sup> antagonists	6	6	6	6	5	5	6
Beta-blockers	5	6	3	2	6	6	3
K <sub>ATP</sub> opener	0	0	0	0	0	0	0
Mean LA size (cm ± SEM)	<4	<4	<4	<4	2.8 ± 0.4	3.2 ± 0.36	4.2 ± 0.19
Mean RA size (cm ± SEM)	<4	<4	<4	<4	<4	3.4 ± 0.14	3.7 ± 0.24
Mean PA pressures (mm Hg ± SEM)	ND	ND	ND	ND	ND	24.1 ± 5.4	36.8 ± 11.1
LVEF (%) ± SEM)	58.1 ± 12.4	60.2 ± 5.4	55.8 ± 3.9	52.1 ± 6.1	63.2 ± 1.6	35.2 ± 3.1	23.1 ± 5.1

DCD = diet-controlled diabetics; IDD = insulin-dependent diabetics; LA = left atrial; LVEF = left ventricular ejection fraction; MI = myocardial infarction; ND = not determined; NIDD = noninsulin-dependent diabetics; PA = pulmonary artery; RA = right atrial.



**Figure 1.** Experimental protocols for study 1. All groups were equilibrated for 30 min in aerobic conditions (37°C). Then the right atrial slices were randomly assigned to one of the study groups (n = 6/group): Aerobic Control = aerobically incubated for the entire experimental time. Ischemia Alone = subjected to 90 min simulated ischemia followed by 120 min reoxygenation. PC = preconditioning with 5 min ischemia followed by 5 min reoxygenation before undergoing 90 min ischemia. Diazoxide = incubation in 0.1 mM of the drug for 10 min before exposure to 90 min ischemia. Glibenclamide = pretreatment in 10 μM of the drug for 10 min before preconditioning was applied.

37°C. The oxygenation of the incubation medium was maintained by a continuous flow of 95% O<sub>2</sub>/5% CO<sub>2</sub> gas mixture to obtain a pO<sub>2</sub> between 25 and 30 kPa and a pCO<sub>2</sub> between 6.0 and 6.5 kPa. The pO<sub>2</sub>, pCO<sub>2</sub> and pH in the incubation medium were monitored by intermittent analyses of the effluent by using an automated blood gas analyzer (Model 855 Blood Gas System, Chiron Diagnostics, Halstead, Essex, United Kingdom) and the pH was kept between 7.36 and 7.45. For the induction of simulated ischemia, the medium was bubbled with 95% N<sub>2</sub>/5% CO<sub>2</sub> (pH 6.8 to 7.0) and D-glucose was removed and the pO<sub>2</sub> was maintained at 0 kPa. In this preparation, tissue injury and viability were assessed but the atrium was not paced and force development was not measured.

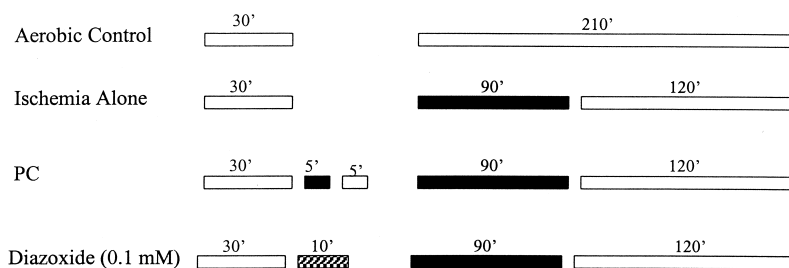
**Solutions.** The incubation medium was prepared daily with deionized distilled water and contained: NaCl (118 mmol/l), KCl (4.8 mmol/l), NaHCO<sub>3</sub> (27.2 mmol/l), KH<sub>2</sub>PO<sub>4</sub> (1 mmol/l), MgCl<sub>2</sub> (1.2 mmol/l), CaCl<sub>2</sub> (1.25 mmol/l), D-glucose (10 mmol/l) and HEPES (20 mmol/l). During simulated ischemia, the substrate D-glucose was removed and replaced with 2-deoxy glucose (10 mmol/l) to maintain a constant osmolarity. The K<sub>ATP</sub> channel blocker glibenclamide was made up to a concentration of 10 μmol in 100 ml K-H solution and the K<sub>ATP</sub> opener diazoxide was dissolved in dimethylsulfoxide immediately before being added into the experimental solutions. All reagents were obtained from Sigma Chemical Co. (Poole, Dorset, United Kingdom).

**Experimental protocols.** After the atrium was sectioned, the preparations were allowed to stabilize for 30 min and then randomly allocated to various protocols. In all studies simulated ischemia was induced for 90 min followed by 120 min of reoxygenation. The drugs were applied to the sections for 10 min after the initial 30 min of stabilization and then removed before ischemia. Two studies were performed:

**STUDY 1.** To investigate whether diabetes influences the protective effect of PC, atrial specimens were collected from four groups of patients: nondiabetics, diet-controlled diabetics (DCD), noninsulin-dependent diabetics (NIDD) on long-term oral sulphonylureas and insulin-dependent diabetics (IDD). Preparations from each group were then randomly allocated to various protocols (n = 6/group) shown in Figure 1.

**STUDY 2.** To investigate the effect of cardiac function on the protection induced by PC, atrial specimens were collected from three groups of patients: with normal left ventricular ejection fraction (LVEF >50%); with moderately impaired function (LVEF 30% to 50%) and with severely impaired function (LVEF <30%). Preparations from each group were then randomly allocated to various protocols (n = 6/group) shown in Figure 2.

In both these studies, PC was induced by a single cycle of 5 min ischemia/5 min reoxygenation, a protocol that we have demonstrated provides maximal protection in this



**Figure 2.** Experimental protocols for study 2. All groups were equilibrated for 30 min in aerobic conditions (37°C). Following this, the right atrial slices were randomly assigned to one of the following study groups (n = 6/group): Aerobic Control = aerobically incubated for the entire experimental time course. Ischemia Alone = subjected to 90 min simulated ischemia followed by 120 min reoxygenation. PC = preconditioning with 5 min ischemia followed by 5 min reoxygenation before undergoing 90 min ischemia. Diazoxide = incubation in 0.1 mM of the drug for 10 min before exposure to 90 min ischemia.

model (19). Preliminary studies (data not shown) had demonstrated that increasing the number of cycles of 5 min ischemia/5 min reoxygenation from one to three does not elicit protection beyond that obtained with a single cycle. In the groups receiving diazoxide and glibenclamide, the drugs were used at a concentration of 0.1 mM and 10  $\mu$ M, respectively. We have shown that these drug concentrations are the minimum effective concentration required to elicit a response and to block PC (7).

**Assessment of tissue injury and viability.** At the end of each experimental protocol, tissue injury was determined by measuring the leakage of creatine kinase (CK) into the incubation medium and tissue viability by the reduction of 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide (MTT) to blue formazan product.

**CK LEAKAGE.** The activity of CK leakage into the medium during the reperfusion period was assayed by a kinetic ultraviolet method based on the formation of NAD (Sigma Catalogue No. 1340-K) at 37°C and the results expressed as U/g wet wt.

**MTT REDUCTION.** At the end of the experimental time, the tissue was loaded into a Falcon conical tube (15 ml, Becton Dickinson Labware, Cowley, United Kingdom) and 2 ml of phosphate buffer solution (0.05 M) containing MTT (1.25 mg/ml, 3 mM at final concentration) was added, incubated for 30 min at 37°C and then homogenized in 2 ml dimethyl sulfoxide (Homogenizer Ultra-Turrax T25, dispersing tool G8, IKA-Labortechnik, Staufen, Germany) at 9,500 rpm for 1 min. The homogenate was then centrifuged at 1,000 g for 10 min and 0.2 ml of the supernatant was dispensed into a 96-well flat-bottom microtiter plate (Nunc Brand Products, Roskilde, Denmark). After this, the absorbance was measured on a plate reader (Benchmark, Bio-Rad Laboratories, Hercules, California) at 550 nm and the results expressed as OD/mg wet wt.

**Statistical analysis.** All data are presented as mean  $\pm$  SEM. All values were compared by two-way analysis of variance with application of a post hoc Bonferroni's test. Statistical significance was assumed at the  $p < 0.05$  level.

## RESULTS

All samples entering the studies completed the applied experimental protocol and were included in the analysis.

**Effect of PC in diabetes (Study 1).** Figure 3A shows that CK leakage was increased to a similar degree by ischemia alone in all study groups. It also shows that PC completely reversed the effect of ischemia in the nondiabetics and DCD groups, so that mean CK leakage values were similar to those in the aerobic control group, but did not have a protective effect in NIDD and IDDM groups. Interestingly, diazoxide, a specific mito  $K_{ATP}$  channel opener, mimicked the protection afforded by PC in nondiabetics and DCD groups but failed to protect the NIDD and IDDM groups. As expected, glibenclamide, a nonspecific  $K_{ATP}$  channel

blocker, abolished the protection of PC in nondiabetics and in DCD and had no effect in the NIDD and IDDM groups. The MTT results shown in Figure 3B are a mirror image of the CK leakage results with PC and diazoxide exhibiting a similar protection in the nondiabetic and DCD groups and no protection in the NIDD and IDDM groups. Overall, the results suggest that changes in mito  $K_{ATP}$  channels in patients with NIDD and IDDM are the most likely cause for the failure to precondition the myocardium. However, the possibility that alterations in signal transduction pathways could also contribute cannot be completely excluded.

**Effect of PC in patients with contractile cardiac dysfunction (Study 2).** Figure 4A also shows that ischemia alone resulted in a significant increase in CK leakage similar in all study groups regardless of the severity of cardiac dysfunction. Importantly, PC was significantly protective; decrease of CK leakage was observed in the groups with LVEF  $\geq 30\%$  but not in the group with LVEF  $< 30\%$ . As observed in study 1, diazoxide mimicked the effect of PC on CK leakage in the groups with LVEF  $\geq 30\%$ . However, in contrast with the absence of protection by PC in the group with LVEF  $< 30\%$ , diazoxide reduced CK leakage in the LVEF  $< 30\%$  group to an extent similar to that seen in LVEF  $\geq 30\%$  groups. The results on MTT reduction shown in Figure 4B were again a mirror image of the CK leakage, suggesting that the cause for the absence of protection by PC in the LVEF  $< 30\%$  group is probably due to alterations in some element(s) of the signal transduction pathway that exclude the mito  $K_{ATP}$  channels.

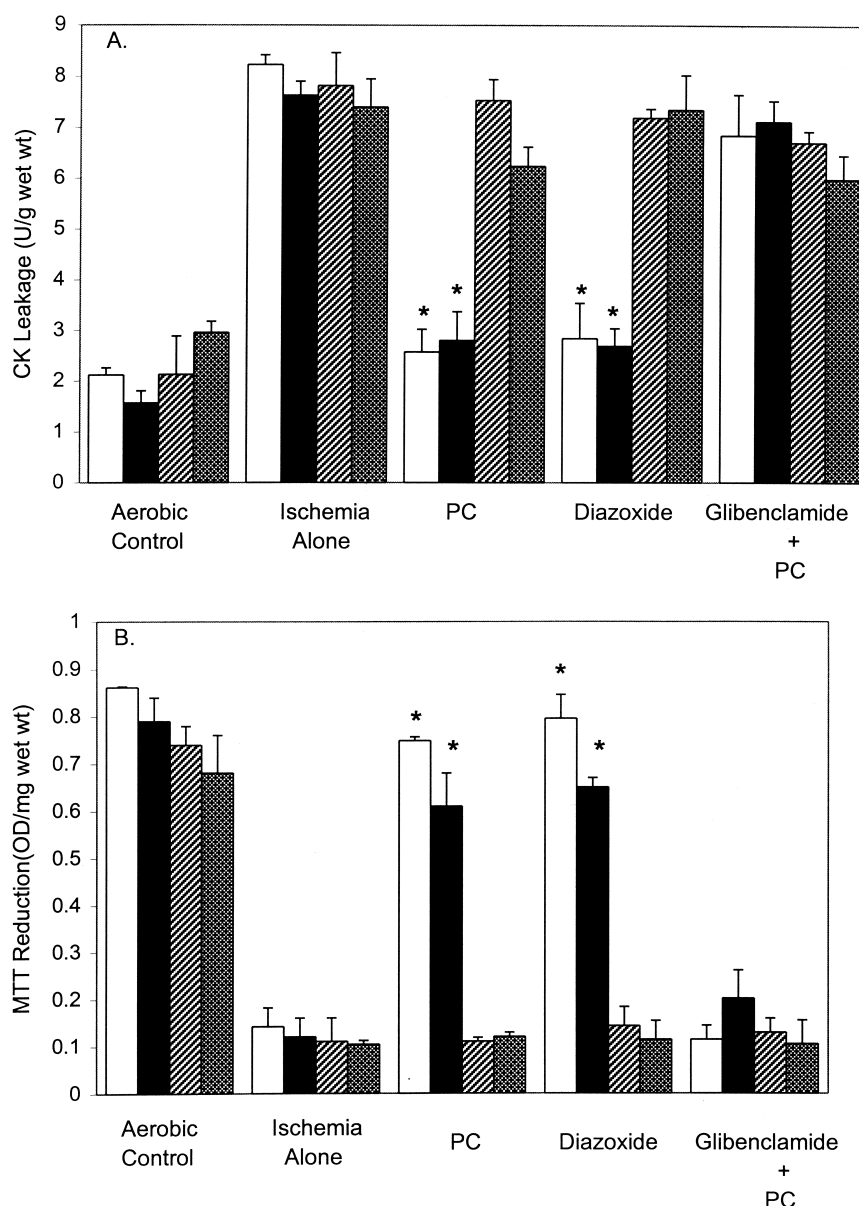
It should be noted that in these two studies the number of patients in each study group was too small for analysis of the possible influence of different clinical characteristics on the effects of ischemia and preconditioning.

## DISCUSSION

The major findings of this study are that myocardium from patients with diabetes and poor cardiac function is not protected from ischemic PC although the injury induced by ischemia/reperfusion is not exacerbated in these conditions. Furthermore, the demonstration that activation of mito  $K_{ATP}$  channels in myocardium from hearts with poor cardiac function mimics the protection induced by preconditioning in the myocardium from hearts with good contractility, but not from patients with diabetes, suggests that the failure to precondition in these conditions is due to alterations of different elements of the signal transduction pathway. Cardiovascular mortality is increased in patients with heart failure (20,21) and diabetes (10) and therefore any myocardial adaptation to ischemia would decrease mortality associated with these diseases. The results of our studies may have important clinical implications and a number of points warrant further discussion.

**Preconditioning and diabetes.** Insulin regulates the balance of energy substrates available to the heart and also regulates metabolism and myocardial perfusion via actions



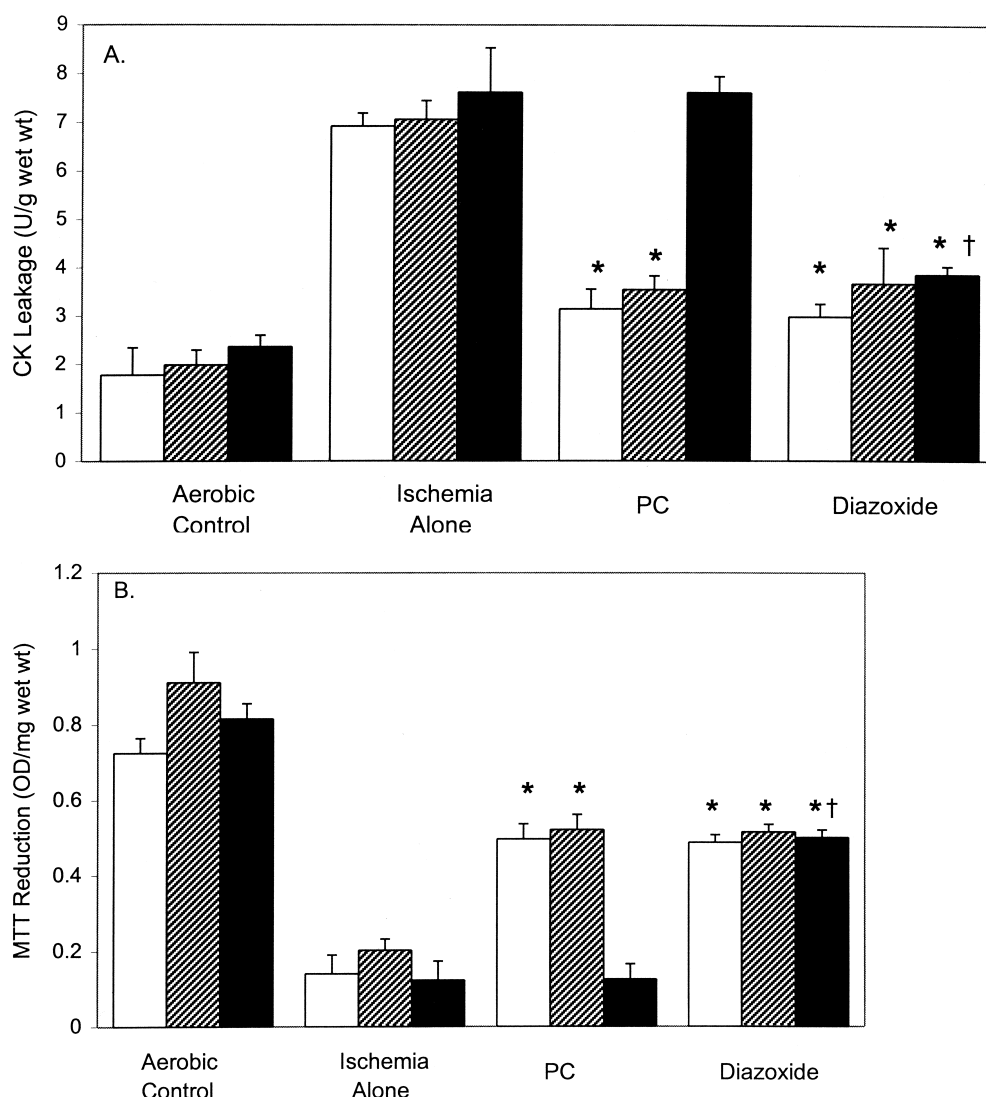


**Figure 3.** Creatine kinase (CK) leakage (A) during the 120 min reoxygenation period (last 120 min in the aerobic control group) and MTT reduction (B) at the end of the reoxygenation period in study 1 (for protocol see Fig. 1). Data are expressed as mean  $\pm$  standard error of mean of six experiments. \* $p < 0.05$  versus corresponding group subjected to ischemia alone. **White bar** = nondiabetics; **black bar** = diet-controlled diabetes; **striped bar** = noninsulin-dependent diabetes; **hatched bar** = insulin-dependent diabetes; PC = preconditioning.

on various intracellular regulatory proteins and messenger systems (22). It is therefore conceivable that diabetes may affect ischemic injury. However, our results show that the diabetic human myocardium is not more sensitive to lethal ischemic injury than the nondiabetic myocardium under the conditions of the present study. Because epidemiological studies have clearly shown that patients with either IDD or NIDD are more prone to develop myocardial infarction and postinfarction complications (10,23), it may be argued that the cause of cardiac complications in diabetics is not the tolerance of the heart to ischemia. The literature is inconsistent with respect to how susceptible the hearts of diabetic animals are to injury from ischemia/reperfusion, and whereas some studies have observed a greater susceptibility

to ischemia/reperfusion injury (15), others have reported no significant effect (24,25). The divergent results may be explained by experimental differences, but if our results in the human atrial myocardium can be confirmed in ventricular myocardium then our attention to reduce cardiac complications in diabetes should be centered in the context of blood components and the vasculature rather than in the own myocardium.

Our studies have demonstrated that PC affords protection of the myocardium from patients with DCD but that this is lost when patients are on long-term hypoglycemics or become insulin dependent. These results are in agreement with those reported by Cleveland *et al.* (16). These investigators showed that myocardium from patients with dia-



**Figure 4.** Creatine kinase (CK) leakage (A) during the 120 min reoxygenation period (last 120 min in the aerobic control group) and MTT reduction (B) at the end of the reoxygenation period in study 2 (for protocol see Fig. 2). Data are expressed as mean  $\pm$  standard error of mean of six experiments. \* $p < 0.05$  versus corresponding group subjected to ischemia alone; † $p < 0.05$  versus corresponding group subjected to preconditioning (PC). **White bar:** left ventricular ejection fraction (LVEF)  $>50\%$ ; **striped bar:** LVEF = 30% to 50%; **black bar:** LVEF  $<30\%$ .

betes on long-term  $K_{ATP}$  blockers also does not precondition in a model of myocardial stunning. However, the same authors also suggested that the myocardium from IDD subjects can be preconditioned, although the protective effect obtained was not to the same extent as that in nondiabetics. Again, these results in the human tissue contrast with the reported experimental results. Liu and co-workers (15) were the first to examine preconditioning in experimental streptozotocin-induced diabetic rats and found that diabetic hearts were more resistant to infarction than normal control hearts and that preconditioning conferred additional protection under in vivo experimental conditions. However, a subsequent study examined the effect of streptozotocin-induced diabetes on the response to ischemia/reperfusion and preconditioning in the isolated rat heart at different stages following its induction (26) and showed that the diabetic heart is more resistant to ischemia/

reperfusion in the early phase of the diabetes (two weeks after onset); but that this protection is lost by four to six weeks. In addition, they observed that the diabetic heart can be preconditioned after six to eight weeks. It must be mentioned that the disparity in the results observed between the two studies could be explained, at least in part, by the differences in the models used. Thus, for example, in the study by Liu and colleagues the model of diabetes is unique in that diabetes was induced by streptozotocin in the neonate and then animals were allowed to grow into adulthood before any intervention was carried out.

These studies do not provide an explanation for such a disparity of results; however, they suggest that abnormalities of mito  $K_{ATP}$  channels may be responsible for the failure to precondition the myocardium of diabetics. Several investigators (27,28), including ourselves (7), have previously demonstrated that mito  $K_{ATP}$  channels are involved in the

protection of ischemic PC. The findings by Smith et al. (29) that  $K_{ATP}$  channels are altered, with a greater outward single-channel current in the ventricular myocardium of diabetic rats, further support this thesis. Clearly, further research is needed to fully elucidate how the alteration of this channel contributes to the failure to precondition the myocardium of diabetics.

**Preconditioning and the failing heart.** Left ventricular hypertrophy and LV chamber dilation are among the compensatory mechanisms of the failing heart. There is experimental evidence that the hypertrophied myocardium is at greater risk from ischemia/reperfusion injury, and it is generally believed that the failing heart is less tolerant to such injury. Our results, however, have shown for the first time that the effects of ischemia/reoxygenation are similar in the failing and nonfailing myocardium. In addition, we have also demonstrated for the first time that the myocardium from hearts exhibiting a LVEF <30% cannot be preconditioned. Our results contrast with those of Cleveland et al. (3) showing that the isolated ventricular trabeculae obtained from patients undergoing cardiac transplantation can be preconditioned. Again, the explanation for this difference cannot be found in the reported experimental studies, and whereas some investigators have shown protection of the failing heart by PC (3), others have observed no effect (30) or even further tissue damage (18). The diversity of results is not entirely surprising because of the lack of uniformity of experimental design and the degree of heart failure we have shown in this study.

The protection observed with diazoxide in the myocardium from hearts with LVEF <30% was commensurate with the protection induced by PC in the myocardium from nonfailing hearts. This supports the thesis that the failure to precondition the failing human heart is not due to an alteration in the response of the mitochondrial  $K_{ATP}$  channel but is caused by abnormalities in other elements of the preconditioning signaling pathway. Considerable evidence indicates that PKC is intimately involved in ischemic PC (31), and the failure to precondition the failing heart may be due to the chronic activation of PKC observed in this condition (32). There are several PKC isoforms, some of which have been involved with PC, and in future studies the type of isoforms and their expression in the myocardium of the failing heart should be investigated. Indeed, if specific PKC isoforms are proved to be responsible for the failure to precondition, then their manipulation could become a therapeutic intervention to reduce myocardial injury in ischemia/reperfusion of the failing heart.

**Possible limitations of the study and clinical implications.** A potential limitation of our study was the use of atrial tissue as opposed to ventricular myocardium, and therefore any extrapolation must be conducted with caution; however, Yellon and colleagues have suggested that PC exerts identical protection in atrial and ventricular myocardium (2). The present study also used atrial tissue to characterize the effects of ischemia and reperfusion in the

failing and diabetic human myocardium. However, atrial and ventricular myocardium possess characteristics of their own that may influence susceptibility to ischemia/reperfusion injury, and as a consequence results from one may not be applicable to the other. Thus the reported differences in the distribution of potassium channels (33,34), which contribute to the characteristic differences between atrial and ventricular action potentials, may determine a different response to ischemia/reperfusion. Undoubtedly,  $K_{ATP}$  channels are present in both atrium and ventricle (33), although their density in both tissues is unknown. It must also be mentioned that the preparation is superfused ("simulated ischemia") as opposed to being arterially perfused, and simulated ischemia is achieved by removal of oxygen and blocking glycolytic ATP production with 2-deoxyglucose. This results in metabolic conditions within the myocardium that may be different from those that occur in the myocardium during clinical ischemia.

Another limitation might be that right atrial appendages were obtained from patients subjected to various medical treatments (e.g., nitrates, beta-blockers, calcium antagonists), which may have influenced ischemia/reperfusion injury and the protection induced by PC. However, it should be emphasized that all medication was stopped the day before surgery when specimens were taken for the study, and that significant effect of the medication was unlikely because all preparations responded to ischemia/reperfusion with a similar degree of injury. The preparation used in this study was not electrically stimulated (i.e., nonbeating) and therefore one should be cautious when extrapolating to the *in vivo* situation.

**Conclusions.** Preconditioning is a potent protective intervention whose use has been advocated in clinical situations such as angioplasty and cardiac surgery. The results of our studies have obvious clinical implications in that PC cannot be beneficial to patients with NIDD or IDD and those with cardiac failure. The results also show that in the failing heart a degree of protection similar to that seen with PC can be obtained by the administration of a selective opener of mitochondrial  $K_{ATP}$  channels, an intervention that is not effective in diabetics.

### Acknowledgments

We thank Professor David Jones for his help with statistical analysis of the data.

---

**Reprint requests and correspondence:** Professor Manuel Galiñanes, Division of Cardiac Surgery, Department of Surgery, University Hospitals Leicester, Glenfield Campus, Leicester LE3 9QP, United Kingdom. E-mail: mg50@leicester.ac.uk.

---

### REFERENCES

1. Carrol R, Yellon DM. Myocardial adaptation to ischemia—the preconditioning phenomenon. *Int J Cardiol* 1999;68:S93–S101.
2. Speechly-Dick ME, Grover GJ, Yellon DM. Does ischemic precon-

- conditioning in the human involve PKC and the ATP-dependent K<sup>+</sup> channel? *Circ Res* 1995;77:1030-5.
3. Cleveland JC Jr., Wollmering MM, Meldrum DR, et al. Ischemic preconditioning in human and rat ventricle. *Am J Physiol* 1996;271:H1786-94.
4. Pain TS, Cohen MV, Downey JM. The mitochondrial K<sub>ATP</sub> channel may be a trigger rather than the end-effector of preconditioning's anti-infarct effect (abstr.). *Circulation* 1999;100:I342.
5. Forbes RA, Steenburgen C, Murphy E. The protective effect of diazoxide is blocked by anti-oxidants (abstr.). *Circulation* 1999;100:I342.
6. Wang Y, Hirai K, Ashraf M. Activation of mitochondrial K<sub>ATP</sub> channel for cardiac protection against ischemic injury is dependent on PKC activity. *Circ Res* 1999;85:731-41.
7. Ghosh S, Standen NB, Galinanes M. Evidence for mitochondrial K<sub>ATP</sub> channels as effectors of human myocardial preconditioning. *Cardiovasc Res* 2000;45:934-40.
8. Kannell WB, McGee DL. Diabetes and cardiovascular risk factors. *Circulation* 1979;59:8-13.
9. Roberts WC. Preventing and arresting coronary atherosclerosis. *Am Heart J* 1995;130:580-600.
10. Kannell WB. Role of diabetes in cardiac disease: conclusion from population studies. In: Zonaraich S, editor. *Diabetes and the Heart*. Springfield, Illinois: Thomas Publishers, 1978:97-112.
11. Hearse DJ, Steward DA, Chain EB. Diabetes and the survival and recovery of the anoxic myocardium. *J Mol Cell Cardiol* 1975;7:397-415.
12. Feuvray D, Idell-Wenger JR, Neely JR. Effects of ischemia on rat myocardial function and metabolism in diabetes. *Circ Res* 1979;44:322-9.
13. Tani M, Neely JR. Hearts from diabetic rats are more resistant to in-vitro ischemia: possible role of altered Ca<sup>2+</sup> metabolism. *Circ Res* 1998;62:931-40.
14. Tosaki A, Pali T, Droy-Lefaix M-T. Effects of ginkgo biloba extract and preconditioning on diabetic rat myocardium. *Diabetologia* 1996;39:1255-62.
15. Liu Y, Thornton JD, Cohen MV, et al. Streptozotocin-induced non-insulin-dependent diabetes protects hearts from infarction. *Circulation* 1993;88:1273-8.
16. Cleveland JC Jr., Meldrum DR, Cain BS, et al. Oral sulphonylurea hypoglycaemic agents prevent ischemic preconditioning in human myocardium. Two paradoxes revisited. *Circulation* 1997;96:29-32.
17. Scheuer J. Metabolic factors in myocardial failure. *Circulation* 1993;87:VII54-57.
18. Dekker LRC, Rademaker H, Vermeulen JT, et al. Cellular uncoupling during ischemia in hypertrophied and failing rabbit ventricular myocardium. Effects of preconditioning. *Circulation* 1998;97:1724-30.
19. Ghosh S, Standen NB, Galinanes M. Preconditioning the human myocardium by simulated ischemia: studies on the early and delayed protection. *Cardiovasc Res* 2000;45:350-9.
20. Franciosa JA, Wilen M, Ziesche S, Cohn JN. Survival in men with severe chronic left ventricular failure due to either coronary heart disease or idiopathic dilated cardiomyopathy. *Am J Cardiol* 1983;51:832-6.
21. Kjekshus J. Arrhythmias and mortality in congestive cardiac failure. *Am J Cardiol* 1990;65:42I-48I.
22. Stanley WC, Lopaschuk GD, McCormack JG. Regulation of energy substrate metabolism in the diabetic heart. *Cardiovasc Res* 1997;34:25-33.
23. Stone PH, Muller JE, Hartwell T. The effect of diabetes mellitus on prognosis and serial left ventricular function after acute myocardial infarction: contribution of both coronary disease and diastolic left ventricular dysfunction to adverse prognosis. The MILIS Study Group. *J Am Coll Cardiol* 1989;14:49-57.
24. Paulson DJ. The diabetic heart is more sensitive to ischemic injury. *Cardiovasc Res* 1997;34:104-12.
25. Feuvray D, Lopaschuk GD. Controversies on the sensitivity of the diabetic heart to ischemic injury: the sensitivity of the diabetic heart to ischemic injury is decreased. *Cardiovasc Res* 1997;34:113-20.
26. Tosaki A, Engelman DT, Engelman RM, Das DK. The evolution of diabetic response to ischemia/reperfusion and preconditioning in isolated working rat hearts. *Cardiovasc Res* 1996;31:526-36.
27. Liu Y, Sato T, O'Rourke B, Marban E. Mitochondrial ATP-dependent potassium channels: novel effectors of cardioprotection? *Circulation* 1998;97:2463-9.
28. Garlid K, Paucak P, Yarov-Yarovoy V, et al. Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP-sensitive K<sup>+</sup> channels: a possible mechanism of cardioprotection. *Circ Res* 1997;81:1072-82.
29. Smith JM, Wahler GM. ATP-sensitive potassium channels are altered in ventricular myocytes from diabetic rats. *Mol Cell Biochem* 1996;158:43-51.
30. Moolman JA, Genade S, Tromp E, Opie LH, Lochner A. Ischemic preconditioning does not protect hypertrophied myocardium against ischemia. *S Afr Med J* 1997;87 Suppl 3:C151-6.
31. Cohen MV, Downey JM. Preconditioning during ischemia: basic mechanism and potential clinical applications. *Cardiol Rev* 1995;3:137-49.
32. Bowling N, Walsh RA, Song G, Estridge T, et al. Increased PKC activity and expression of calcium sensitive isoforms in the failing human heart. *Circulation* 1999;99:384-91.
33. Heidebuchel H, Vereecke J, Carmeliet E. Three different potassium channels in human atrium: contribution to the basal potassium conductance. *Circ Res* 1990;66:1277-86.
34. Amos GJ, Wettwer E, Metsger F, Li Q, Himmel HM. Differences between outward currents of human atrial and subepicardial ventricular myocytes. *J Physiol* 1996;491:31-50.